Carbonyls in Oxidizing Fat. VII. A Comparison of Methods of Isolating Monocarbonyl Compounds

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Abstract

Comparison of quantitative methods for the isolation of aldehydes from lard oxidized at 23C showed an extreme sensitivity of precursors to isolation conditions. Differences existed in the quantity and class compositions of the monocarbonyl compounds isolated. Acid conditions caused considerable breakdown of alkanal and alk-2-enal precursors. Steam distillation (100C) released alkanals, alk-2-enals, and alk-2,4-dienals. The mild Girard T method apparently produced a small amt of alk-2-enals from precursors, but except in the initial stages isolated the lowest amounts of aldehydes. Complete evaluation of the method for isolation of free aldehydes was not possible because alkanals were released from precursors during the vacuum distillation procedure (50C). The Schwartz (dilute phosphoric acid), Girard T, and vacuum distillation methods isolated similar amounts of alk-2,4-dienals. Curious changes in relationships, possibly indicative of oxidation stages, were observed as autoxidation progressed.

METHODS USED for the isolation and determination of carbonyl compounds from oxidized lipids differ greatly in the severity of conditions. It has been generally recognized that an oxidized fat is in a dynamic state and that mild treatments are essential for the purpose of isolating the free carbonyl compounds. Since it is the free carbonyl compounds that produce the flavors, this is an important consideration. Carbonyl compounds separated by a distillation or volatilization procedure at low temps and pressures might be expected to be representative of those existing in a free state. For simplicity and rapidity, as well as for the removal of nonvolatile carbonyl compounds, methods have been devised which permit direct reaction of a carbonyl reagent with the oxidized lipid. Among these are Begemann-de Jong (1), Schwartz (26), Henick (12), Pool and Klose (22), and Girard T (11) methods. Except for the Pool and Klose method (16,22), quantitative reaction with moncarbonyl compounds is generally obtained. However, under the necessary conditions the Girard T (11) reagent reacts poorly with methyl ketones and presumably other ketones. The chief difference in these methods lies in the kind and degree of acidity used. The Begemann-de Jong method (1) uses 2 N hydrochloric acid. Dilute phosphoric acid is used in the Schwartz method (26) with a resulting pH of about 2.0. The Henick method (12) for determination of total carbonyl function uses trichloroacetic acid and heat. The Girard T (11) reaction has a pH of about 5.0, and as modified uses no acid. The Pool and Klose method (22) proceeds on hydrated alumina under nearly neutral conditions. The use of acid has been generally considered to cause carbonyl formation (18) from primary oxidation products. Strong cation exchange resin in the Girard T reaction has been ob-

served to have such an effect (9,28). However, Keeney (14), in a review of fundamental autoxidation reactions, concluded that acids would be expected to convert hydroperoxides to triols and not involve a conversion to carbonyl compounds. Furthermore, Schwartz et al. (26), in an evaluation of their carbonyl method, stated that hydroperoxides disappeared and that linoleate hydroperoxide was not converted into monocarbonyl compounds. However this may be, the statement (17) has been repeatedly made that carbonyl compounds determined in the presence of hydroperoxides are meaningless unless the hydroperoxides are first removed by reduction. Keith and Day (16) modified the Pool and Klose method, and concluded that the method isolates free aldehydes, although not quantitatively (1,16).

Gaddis, Ellis, and Currie (11) recently developed a modified Girard T reagent method which was shown capable of reacting quantitatively with aldehydes in the presence of fat. The performance of these quantitative monocarbonyl methods in the presence of primary oxidation products has been largely undetermined. The purpose of this work was to compare these methods for the isolation of free monocarbonyl compounds (aldehydes) from autoxidized lipids.

Experimental

Solvents, Reagents, and Materials

Solvents, such as carbon tetrachloride, benzene, and tertiary butyl alcohol, were rendered carbonyl free (11) by treatment with 3 g trichloroacetic and 4–5 g 2,4-dinitrophenylhydrazine per liter, refluxing 1 hr and distillation. The carbonyl content of primary and secondary alcohols was reduced to a minimum by a similar treatment. Hexane was purfied by the method of Hornstein and Crowe (13). Lard was rendered a minimum length of time at about 120C and filtered through cheesecloth into cans which were vacuum packed and stored at −30F until used. Two batches of lard (A and B) were employed in this study.

Methods

Vacuum distillations were conducted as described by Lea and Swoboda (18) except the distillation was continued for 50 min instead of 30 min. This was considered necessary because the ultimate vacuum attained was 0.2 mm instead of 0.01 mm. The Girard T determination was made as described by Gaddis, Ellis, and Currie (11). The Schwartz method for monocarbonyl compounds was carried out according to Schwartz, Haller, and Keeney (26). Steam distillation at atmospheric pressure was performed by the method of Gaddis, Ellis, and Currie (6). The Begemann-de Jong determination was made as originally described (1). Henick values for total carbonyl were determined according to Henick, Benca, and Mitchell (12). All determinations on an oxidized sample were set up simultaneously. Duplicate determinations were made for all monocarbonyl isolations. Moncarbonyl 2,4-dinitrophenylhydrazones obtained by direct reac-

tion procedures were freed of traces of fat by the ion exchange resin method of Schwartz et al. (25).

Autoxidations of lard were carried out on thin layers at 23C. During this process the lard was mixed daily. Except as otherwise specified determinations were made on 10.0 g of sample and results as absorbance values are calculated on that basis. All absorbance values represent the amount of hydrazones in 100 ml of carbon tetrachloride. Isolated 2,4-dinitrophenylhydrazones of monocarbonyls were separated by paper chromatography into classes and individual compounds as required by previously reported methods and applications (2,3,7–10,27). In screening monocarbonyl derivatives for high mol wt compounds the vaseline-aqueous methanol paper chromatographic method of Ellis, Gaddis, and Currie (2) was modified. Whatman No. 3 paper strips 18 in. long impregnated with 7% Vaseline and spotted with the mixture were irrigated with 9–1 (v/v) methanol-water overnight. With this system lower aldehydes concentrated at the top and C_{12}-C_{20} aldehyde derivatives were separated. Peroxides were determined on 0.200 g samples according to the method of Kenaston et al. (15) and expressed as milliquivalents per 1,000 g. Statistical analysis of total monocarbonyl values was made by analysis of variance and application of Duncan's Multiple Range test.

Results and Discussion

Preliminary studies indicated wide progressive differences in monocarbonyls isolated by the Schwartz, steam distillation, and Begemann-de Jong methods. Amts of compounds separated increased in the order listed. Vacuum distillation and Girard T methods generally gave lower values than the Schwartz method, but there was considerable variation in relationships. Differences appeared to depend on the degree and conditions of autoxidation. It was clear that a series of careful studies would be required to establish the significance of the noted variations. The autoxidation of mildly rendered lard at 23C in the dark, and under artificial and ultraviolet light, was examined.

Vacuum Distillation and Schwartz Method

A succession of 15 autoxidation experiments has shown a definite reproducible pattern in the differences between amts of monocarbonyl compounds isolated. There were at least two stages during oxidation representing a different relationship between vacuum distillation and Schwartz monocarbonyl values. Initially, Schwartz values exceeded vacuum distillation values at a progressively greater rate. At variable peroxide levels above a peroxide value of 50, the formation of vacuum distillation monocarbonyls accelerated to a faster rate than the Schwartz values. With advanced oxidation the two values tended to become similar, and in extreme cases vacuum distillation exceeded the Schwartz. Twelve of the experiments were statistically analyzed. In 76 multiple comparisons differences between Schwartz and vacuum distillation were 81.7% significant, and Girard T and vacuum distillation 44.7% significant. There was a reversal in relationships between Girard T and vacuum distillation values. Relationships from peroxide 0–50 were 46.7% significant, peroxide 50–160 were 11.1% significant, and peroxide 160–366 were 100% significant. This data was combined by averaging at peroxide intervals of 10. Comparisons not significantly different were assigned the same value by averaging. The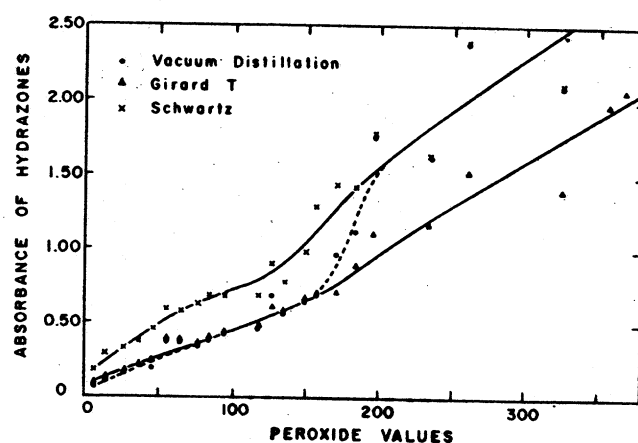 resulting summary is shown in Figure 1. This agrees, in general, with the patterns shown by individual experiments. The Schwartz method quite evidently breaks down some type of autoxidation-formed precursor. The differences present over the interval of peroxide 15 to 35 at which rancidity usually appears in lard could be very significant in flavor evaluation. The overtaking of Schwartz values by the vacuum distillation values at higher oxidation levels may mean a change in the type of monocarbonyl precursors present. Vacuum distillation values which have been generally assumed to represent the free volatile aldehydes were less than 1% of the total determinable carbonyls as measured by the method of Henick. If non-volatile monocarbonyl compounds are present the relationships noted would be affected, since the Schwartz method would isolate such compounds.

Nonvolatile Monocarbonyl Compounds

High mol wt aldehydes bound to glycerol have been observed in relatively abundant amts in some fats (20,21,23). These compounds are hydrolzed by the Schwartz and Begemann-de Jong methods with the resulting isolation of hydrazone derivatives of the aldehydes. The Girard T reagent apparently does not hydrolyze these enol-ether bound aldehydes as judged by direct comparisons with the Schwartz method on appropriate fats. Reaction of fresh, mildly rendered lard on the Schwartz reaction column yielded monocarbonyl hydrazones which upon screening by the Vaseline-aqueous methanol paper partition system showed only the barest traces of C_{16} and C_{18} saturated aldehydes. The comparisons therefore are not complicated by bound nonvolatile aldehydes.

Girard T and Schwartz monocarbonyl compounds from oxidized lard, when examined, did not reveal aldehydes of higher carbon number than C_{12}. Studies of a variety of other oxidized fats (17,19,21,28) have indicated that the moncarbonyl products of autoxidation are volatile. The comparisons are accordingly not influenced by nonvolatile aldehyde oxidation products.

FIG. 1. Monocarbonyl compounds. Summary of statistical analysis.

TABLE I

Comparison of Aldehyde Recoveries from Lard

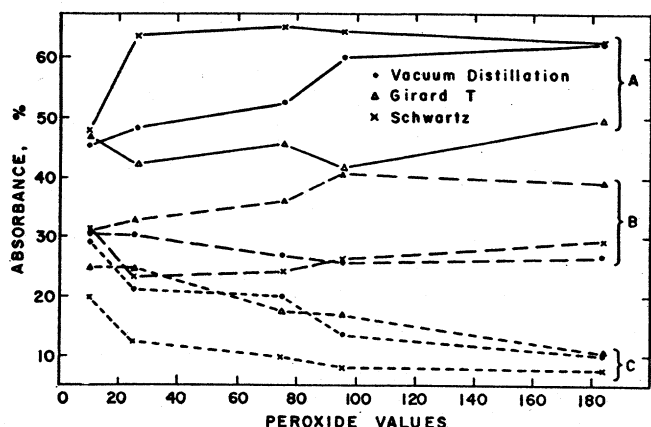|	Vacuum distillation	Girard T	Schwartz	Steam distillation	Begemann-de Jong
Alkanal C_5	1.797	2.098	2.018		
Alkanal C_6	1.868	1.810	1.745	1.860	1.600
Alkanal C_7	1.472	1.406	1.431		
Alkanal C_9	1.413	1.245	1.245	1.430	1.450
Alk-2-enal C_7	2.186	2.085	2.115	2.050	1.880
Alk-2-enal C_8	2.961	2.960	2.825	2.900	
Alk-2,4-dienal C_8	2.825	2.610		2.827	
Alk-2,4-dienal C_{10}	1.670	1.630	1.770	1.537	1.780

FIG. 2. Proportions of monocarbonyl classes. Lard A, 23C in dark. A, alkanal; B, alk-2-enal; C, alk-2,4 dienal.

Vacuum Distillation and Girard T Methods

As shown in Figure 1, Girard T monocarbonyl values in the initial oxidation stage were somewhat higher than the vacuum distillation values. In a second stage which occurred usually after a peroxide of 60, the vacuum distillation values equaled and then exceeded the Girard T values at an increasing rate.

The abrupt change in relationship between vacuum distillation and Girard T values in the second stage might be considered remarkable. Since the compounds involved were aldehydes, the differences could not be due to unequal quantitative capabilities of the two methods. This seems assured by the careful work that has been done in evaluation of these methods (11,18, 24,26, and the data in Table I). It would appear that the aldehydes representing the differences between vacuum distillation and Girard T are not present in a free state in the oxidized fat. Fritsch and Deatherage (5) obtained volatile hydroxyalkyl hydroperoxides from autoxidized oleate and postulated that these compounds could be formed from dihydroperoxides resulting from secondary oxygen attack of the initial hydroperoxide. The presence or formation in the oxidized fat of a volatile precursor, inert or otherwise unavailable to the Girard T reagent, but which reacts with acidic 2,4-dinitrophenylhydrazine to form hydrazones might account for the difference. Fritsch and Deatherage were uncertain whether the volatile peroxides were present in the oxidized oleate or formed in the distillate. However, results were similar when vacuum distillates were reacted with Girard T reagent on the Schwartz column or with acidic 2,4-dinitrophenylhydrazine. The formation of aldehydes during the vacuum distillation procedure is supported by the observations of Lea and Swoboda (18). These investigators noted that continuation of the vacuum distil-
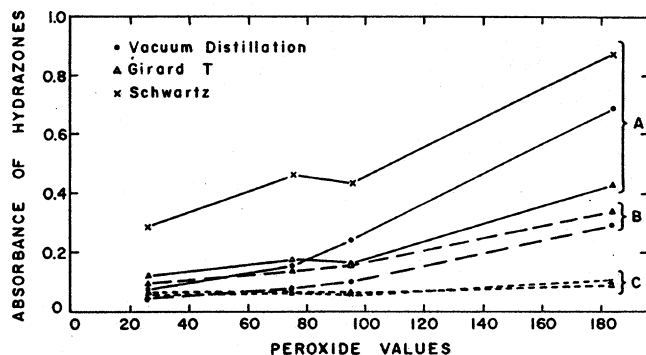

FIG. 3. Absolute amounts of monocarbonyl classes. Lard A, 23C in dark. A, alkanal; B, alk-2-enal; C, alk-2,4 dienal.
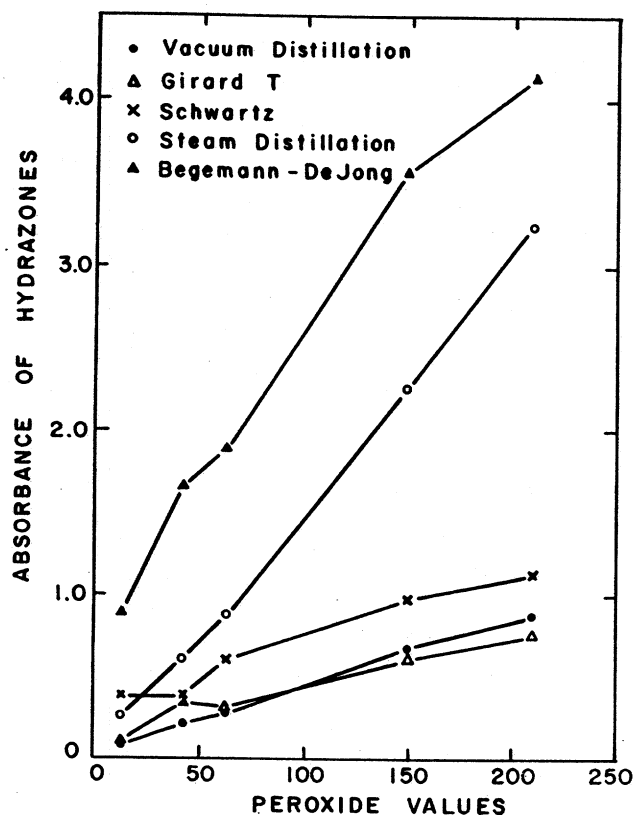

FIG. 4. Monocarbonyl compounds. Lard B, 23C artificial light.

lation beyond the period necessary for quantitative recovery of free aldehydes yielded appreciable amts of volatile carbonyls. Therefore, there appeared to be some formation or release of volatile carbonyls under the mild conditions of heating at 50C under vacuum. Similar results were obtained in this study; for instance, a lard with a peroxide value of 73 had volatile monocarbonyl values of 1.06, 0.098, and 0.065 in successive 30-min periods of vacuum distillation. The 20-min longer vacuum distillation period used in this work undoubtedly influenced the variable relationships between Girard T and vacuum distillation. However, this was a matter of degree only, since the formation of volatile aldehydes must have taken place throughout the distillation. Whether this is due to the mild warming or to a spontaneous formation of aldehydes remains to be determined. There is much that is not understood about the origin of these compounds. At all stages of oxidation, Girard T and Schwartz methods isolated additional quantities of aldehydes from the vacuum distilled fat.

Although a true baseline for free aldehydes is lacking, the data indicated that both the Girard T and vacuum distillation methods break down precursors. The vacuum distillation procedure showed a greater response in this respect with oxidation. The Girard T monocarbonyls had higher absorbance maxima which indicated more unsaturation. However, this might merely indicate a higher proportion of saturated aldehydes isolated by the Schwartz and vacuum distillation procedures. Fritsch and Deatherage obtained saturated aldehydes from the volatile hydroperoxides they described.

Amounts of Monocarbonyl Classes

The monocarbonyl derivatives were separated by paper chromatography into classes to evaluate the observed differences between methods. The data shown in Figure 2, which was a typical result, indicate that
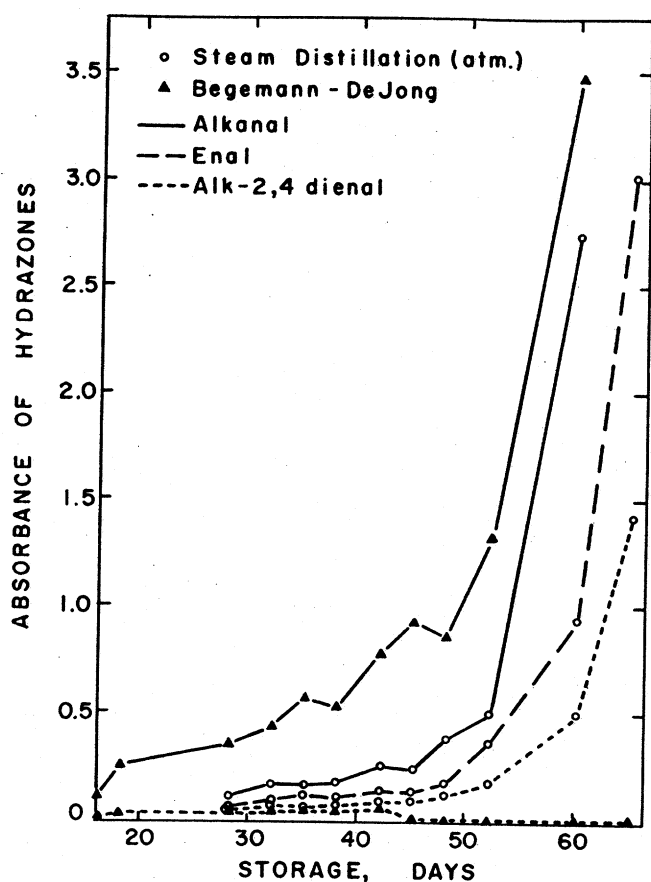
FIG. 5. Absolute amounts of monocarbonyl classes: Lard B, 23C in dark.

FIG. 6. Effect of heating (165C) on monocarbonyl compounds. Lard B, 23C artificial light.

class composition differed widely. Although similar initially, a rapid change took place with oxidation. The greatest differences were in the alkanal class. With advanced oxidation ("second stage") Schwartz and vacuum distillation monocarbonyls tended to become similar in class composition. There was a large and particularly significant difference in enal percentage between the Girard T method and vacuum distillation and Schwartz methods. Over the interval that Girard T and vacuum distillation monocarbonyl values paralleled and were similar, there were increasing differences in alkanal and enal composition.

Absolute values for the monocarbonyl classes shown in Figure 3 illustrate further the basic differences between the Schwartz, Girard T, and vacuum distillation. The greatest differences were in the amts of alkanal, and resulting curves were similar in general form to the total monocarbonyl curves. There were substantial and rather constant differences between Girard T and vacuum distillation enal values. Schwartz enal values, which are not shown, were the highest. Dienal values were identical and increased very slowly with oxidation. Only the Schwartz and Girard T dienal values were plotted. This class of compound is either extremely labile when formed in the oxidizing fat or their parent hydroperoxides are present in small amts or stable. It is well known that heating an oxidized fat produces large amts of 2,4-dienal compound (8,10,27).

Results indicate that the Schwartz method decomposed both saturated and enal aldehyde precursors. The Girard T method appeared to break down small amounts of alk-2-enal precursors. However, the true relationship between Girard T method isolates and free monocarbonyl compounds was obscured by the
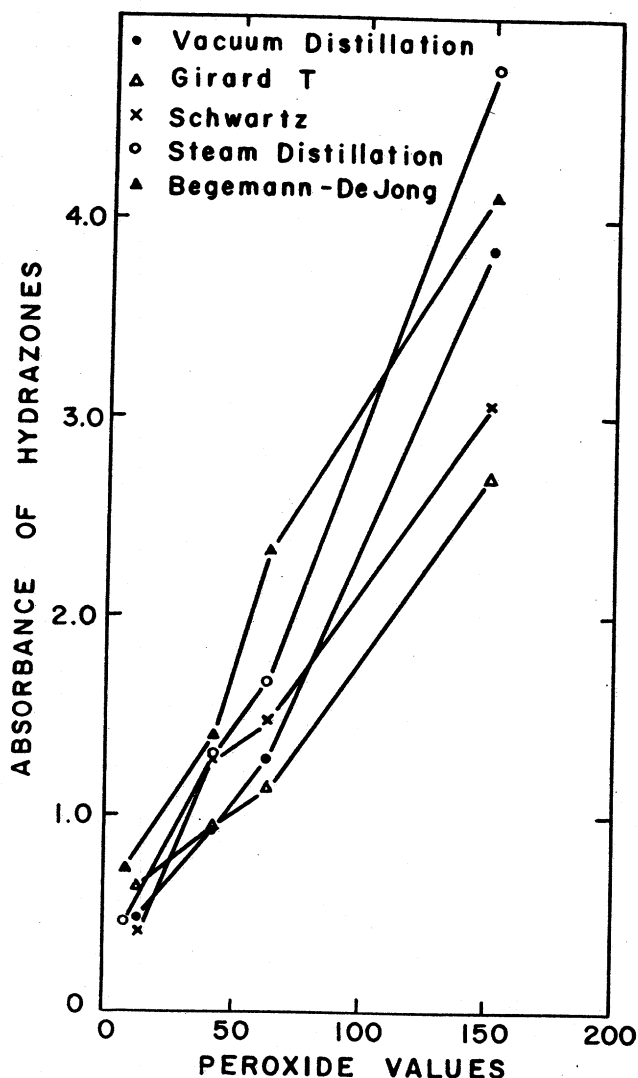
formation of volatile aldehydes during the vacuum distillation procedure. The compounds formed appeared to be mostly saturated aldehydes. Lea and Swoboda (18) noted that carbonyls released during vacuum distillation were saturated aldehydes. The results in Figures 2 and 3 show large differences in alkanal and enal contents of Girard T and vacuum distillation monocarbonyls during the initial oxidation stage when differences between the total quantities were small or not significant. The two methods are therefore giving different results even in this area.

Steam Distillation and Begemann-de Jong Method

The effect of moderate heat (100C) and strong mineral acid is shown in the comparison of all five methods shown in Figure 4. A breakdown of absolute amts of the monocarbonyl classes isolated by the two most vigorous methods in another experiment is shown in Figure 5. The compositions of the steam distillation monocarbonyls were characterized by higher enal and dienal percentages. The Begemann-de Jong enal curve, omitted for clarity of chart, was higher than steam distillation values. The Begemann-de Jong aldehydes gave an abnormal class separation. This was indicated by a fourth spot adjacent to the alkanals which had the maximum of a saturated class. The unknown was not identified and was included in the calculations as alkanal class.

The Decomposition of Monocarbonyl Precursors

Monomeric hydroperoxides, the primary products of autoxidation, are considered the precursors of the monocarbonyl compounds in autoxidized fat. The aldehydes isolated and identified are those predicted by the scission of parent hydroperoxide isomers (9,10). Hydroperoxides are labile and highly reactive, and direct measurement of free aldehydes in their presence has been considered questionable (17). Schwartz et al. (26) showed that, in the dilute phosphoric acid-celite-2,4-dinitrophenylhydrazine reaction method, hydroperoxides disappeared. These investigators, on the basis of experiments with methyl linoleate hydroperoxides, concluded that no monocarbonyls were produced in the Schwartz column reaction. However, significant differences related to autoxidation have been shown in this investigation to occur between the Schwartz and volatile monocarbonyl compounds during the early stages. The observed differences may be due to the decomposition of other hydroperoxides or precursors. Also it should be considered that a pure preparation of hydroperoxides may not be the same as an oxidized fat system. A change occurred with advanced oxidation since volatile monocarbonyls equaled and frequently exceeded those isolated by the Schwartz method.

Heating at 165C breaks down primary oxidation products, greatly increases the monocarbonyl compounds, and modifies their composition. Heating autoxidized lard under N_2 (15 min at 165C) produced the results shown in Figure 6 (as compared with Figure 4). It is evident that the treatment narrowed differences. This would have to be due to the decomposition or decrease of precursors.

The evidence presented shows interesting and significant differences between the monocarbonyl isolation methods examined. This is unquestionably influenced by the carbonyl precursors present, which in turn might be related to the degree and course of autoxidation. It should be considered that the range of autoxidation conditions used in these experiments was purposely narrow; and other conditions, environments and substrates might give other relationship patterns.

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